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BIOLOGICAL BULLETIN.

FORM REGULATION IN CERIANTHUS.

I. The Typical Course of Regeneration.

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Introduction.

During the year 1902–1903 it was my privilege to spend several months at the Zoölogical Station in Naples, as holder of the Smithsonian table. I take this opportunity to express my great indebtedness both to the Smithsonian Institution for the grant and to Professor Dohrn and all other members of the staff of the Zoölogical Station. A part of my time at Naples was devoted to the study of regeneration and other regulative processes in the Cerianthidæ, and an account of these observations and experiments is begun in the present paper.

So far as I am aware the only work upon regulation in *Cerianthus* is that of Loeb.[1] A review of this work is unnecessary at this time since the various points will be discussed in connection with my own observations as occasion arises.

My observations and experiments upon the Cerianthidæ fall into a number of groups, and, since they are somewhat extended, the account of the subject will be divided in a corresponding manner. In the present paper the usual "normal" course of regeneration resulting in a perfect animal is described. Later the problem of experimental control of regulation will be taken up, then variation and abnormalties in regulation and the factors concerned in their production.

The Normal Animal.

It is necessary to call attention to a number of the features of the normal anatomy and habits before proceeding to the description of the regenerative phenomena.

[1] Loeb, J., "Untersuchungen zur Physiologischen Morphologie der Thiere," I., Wurzburg, 1891.

Cerianthus solitarius, the species which formed the subject of most of the experiments, is considerably smaller than *C. membranaceus*. Owing to the varying degrees of distension and contraction accurate measurements of the form are difficult to obtain. A considerable number of specimens were measured when apparently fully extended and the body distended with water. These were all among the larger specimens, for the smaller individuals were discarded in nearly all cases. These measurements are of course only approximate and serve merely to indicate the general proportions of the specimens used for experiment. Under other conditions of contraction or distension these same individuals possess very different proportions. In all cases a single individual was measured repeatedly at intervals and the maximum measurements taken as representing complete extension. The following table presents a few such measurements of different individuals, the measurements being given in millimeters :

Length of Body.	Length of Marginal Tentacles.	Length of Labial Tentacles.	Diameter of Disc.	Diameter of Body in Œsophageal Region.	Diameter of Body Near Aboral End.
90	30–35	12–15	12	7	7
95	25–30	12–15	12	7	5
60	20	9–12	10	6	4.5

The specimens used were between these limits of size. A comparison of the measurements of the three individuals shows that the smaller specimen possesses different proportions from the larger, *i. e.*, its transverse diameters are relatively greater as compared with the length than those of the larger specimens. In other words, after the individuals reach a certain size further increase is chiefly an increase in length. Without giving the figures at this time to prove this point, since it will be taken up later in connection with the discussion of morphallaxis, it may be said that this difference in proportion between small and large specimens is of general and probably universal occurrence in *Cerianthus*. Smaller specimens are always relatively thicker than large ones.

In general form the body is nearly cylindrical, expanding orally to form the disc and tapering slightly posteriorly. At the aboral end is a small pore which under certain conditions permits

the exit of water. In the expanded condition the disc possesses the form of a broad shallow funnel extending from the base of the marginal tentacles to the margin of the mouth and continued aborally in the œsophagus. The mouth is slit-like in form with one siphonoglyph or gonidial groove at one end of the slit. The disc is marked with radiating lines, slightly depressed, which correspond to the lines of attachment of the mesenteries beneath the surface: these continue aborally in the œsophagus. The œsophagus extends aborally from the disc about $\frac{1}{6}$–$\frac{1}{5}$ the length of the body when the animal is fully extended.

The marginal tentacles, as their name implies, are borne upon the margin of the disc, usually in about three rows, the number varying in grown specimens from about 41 to 71. About the margins of the mouth are the shorter labial tentacles which are fewer in number than the marginal tentacles, and form only a single circle.

The body appears brownish in color, but upon close examination is found to be marked with light longitudinal stripes or lines of varying width, some of which extend the whole length of the body while others are shorter. These are in reality merely unpigmented areas between the stripes of brown pigment. The color of the marginal tentacles is in general effect lighter than that of the body, but they are marked by transverse bands of dark pigment. The labial tentacles are brownish and usually unstriped. The disc and œsophagus in large, apparently old specimens are dark brown without definite striping.

As regards the internal anatomy certain points are of interest in this connection. It has long been known that the arrangement of the mesenteries in the Cerianthidæ differs in some respects from that in the other Actinozoa. In the œsophageal region all mesenteries extend from the body-wall to the œsophagus and thus divide the enteron of this region into a series of longitudinal radiating chambers which open into the enteron aborally. At the oral end each of these intermesenterial chambers opens into the cavity of a single marginal tentacle; thus the marginal tentacles are always equal in number to the intermesenterial chambers. The labial tentacles, while corresponding in position to intermesenterial chambers, are fewer in number.

Aboral to the œsophagus the inner margins of the mesenteries hang free in the enteron and bear the mesenterial filaments. A single pair of very short mesenteries at that end of the mouth where the siphonoglyph is situated are known as the directives. The next mesentery on each side of these extends almost to the aboral end of the body. From this point to right and left the mesenteries decrease in length, following a definite, rather complex law which need not be discussed here. On the side opposite the directives, at the opposite angle of the mouth are the shortest mesenteries, with the exception of the directives; these do not extend far aboral to the œsophagus. It is in this region that all new mesenteries are added, *i. e.*, the region of growth is opposite the directives. Thus, proceeding from the directives to the right and left around the body the mesenteries are successively younger. Each pair of new mesenteries appears between the members of the last preceding pair formed, thus separating them. Corresponding to the formation of new intermesenterial chambers new tentacles appear in this region. It is usually possible to find at this point in the normal animal one or two pairs of tentacles much smaller than the others and in process of growth. Corresponding to the number and arrangement of the mesenteries there is one unpaired marginal tentacle over the chamber between the directive mesenteries and known as the directive tentacle. It is usually somewhat thicker than the other tentacles since the space between the directives is greater than that between other mesenteries. The other tentacles are paired right and left.

In *Cerianthus solitarius* the greater number of the mesenteries about the whole circumference of the body do not extend aborally far beyond the œsophagus. Only certain mesenteries extend further, to end at various levels according to their position. This is also true of *Cerianthus membranaceus.*

The muscles of the body-wall consist of a heavy layer of powerful longitudinal muscles which decreases slightly in thickness toward the aboral end. These are the chief muscles of the body, circular muscles being absent, and tentacles, disc and œsophagus possessing only a slight muscular development.

As is well known, the Cerianthidæ are found imbedded in the

sand with the oral end and tentacles protruding. In this position they secrete about the body a mass of tenacious slime in which sand-grains and other foreign bodies become imbedded, the whole forming a tube into which the animal may withdraw. Loeb has given an interesting account of the geotactic reactions of these animals and my incidental observations upon this point confirm his. He has also described a number of experiments concerning the external conditions which determine the tube-formation.

When specimens are kept in aquaria without sand they creep about to a considerable extent, often climbing the sides. When left undisturbed they usually orient themselves as Loeb has noted, so that the oral end of the body is directed upward, even if this position necessitates the bending of the body at right angles. In the jars they secrete a considerable amount of slime and often form tubes along the sides or bottom, in which they remain for a longer or shorter time. When handled or otherwise irritated, and especially when cut, the secretion of the slime is especially rapid.

When undisturbed, the body and tentacles are usually more or less distended with water and the body-wall is always tense. Indeed, as will be shown later, complete extension of the body and erection of the tentacles is impossible without internal water-pressure, *i. e.*, without water in the enteron. If the body of a distended animal is opened quickly by a small cut the water issues with considerable force, and when an individual is made to contract rapidly by sudden stimulation the water squirts from the aboral pore with great force. The inability of the animal to extend to its full length without the aid of water-pressure is due to the absence of circular muscles in the body-wall. Extension is passive, not active. The longitudinal muscles are powerful and under strong stimulus the body may be torn apart if the ends are fastened.

It was found that the animals could be kept alive for months without other food than the small forms and organic particles which the water might contain, and in the present series of experiments no attempt was made to give them food. Of course in the early stages of regeneration and throughout many of the experiments the pieces were unable to take food; moreover, the

growth resulting from an abundant food supply constitutes in any case a complicating factor in the analysis of various phenomena of form regulation. In such experiments as permit the taking of food a complete analysis of the phenomena would include studies of the effect of abundant food supply, but previous experiments along this line indicate that the results in the lower animals differ only in degree and not essentially in kind with the presence or absence of food.

Four species of *Cerianthus* were employed for experiment, viz., *C. solitarius*, *C. membranaceus* and two smaller undetermined species, one of them almost completely colorless. It was soon discovered, however, that *C. solitarius*, a very common form in the Bay of Naples, was more favorable than the other forms on account of size, coloration of body and abundance. My attention was therefore devoted chiefly to this species, though the other forms, and especially *C. membranaceus*, were used for comparative study.

THE COURSE OF REGENERATION.

The cut pieces were isolated in dishes which were placed in aquaria supplied with flowing water. During the earlier stages of regeneration the pieces showed little tendency to creep out of dishes, but later it was necessary in some cases to cover the dishes with netting to prevent escape.

In *Cerianthus* the course of regeneration is complicated in many cases by various factors, such as the form of the pieces, the internal water pressure, etc. The simplest cases are those in which the body is divided by a transverse cut into two pieces, or a piece is removed by two transverse cuts. In such cases a nearly cylindrical piece is obtained which regenerates at the cut end or ends. Such pieces are best fitted for the study of the "typical" course of regeneration at the two ends, and since a knowledge of this is important as a preliminary to the study of experimental control of regeneration this paper is devoted to a description of the phenomena concerned in such cases.

A piece cut from the middle region of the body (*e. g.*, between the lines *aa* and *bb*, Fig. 1) will serve as an example.

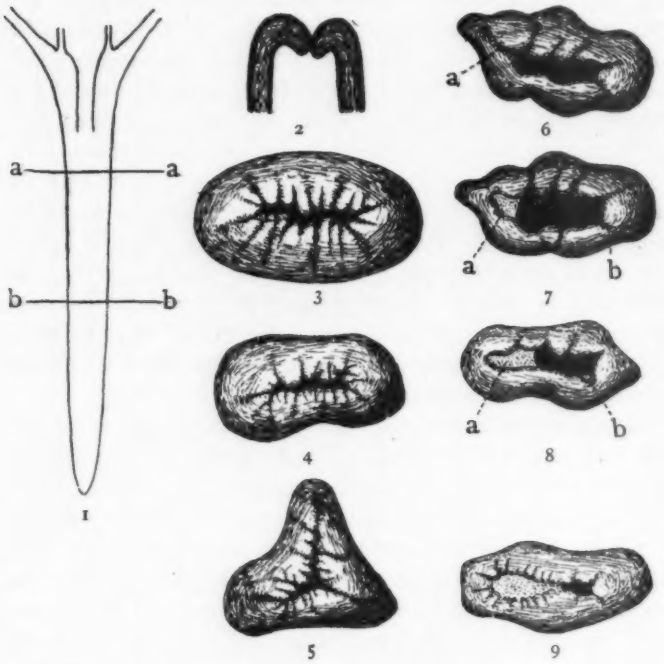
The Immediate Effects of the Operation.

Individuals which were in good condition and well extended were chosen and the cuts were made rapidly with sharp scissors. All parts of the body contract strongly in consequence of the cut, and of course total collapse of the piece occurs, owing to the escape of the water from the enteric cavity. Within a few moments the piece may relax somewhat from the extreme condition of contraction, but does not attain anything like its original length. Placed in the jar it lies on the bottom, and the weight of the tissues causes it to become more or less flattened. The piece has no power to retain its cylindrical form, though the mesenteries and mesenterial filaments, especially in pieces cut from the oral half of the body, partly fill the enteron and so cause the piece to retain a more or less rounded form. The body-wall is opaque in these pieces, while in normal specimens distended with water it is slightly translucent. The opacity is due simply to its greater thickness in the absence of the tension caused by internal water-pressure.

Within a few moments after section the cut edges at the two ends of the piece begin to bend or roll inward, and in an hour or two this inrolling has proceeded so far that the cut edges are no longer visible from the ends and the opening is almost completely closed by the inrolled portions. In Fig. 2 a longitudinal section through the oral end of such a piece is shown, the ectoderm and entoderm being indicated by full black lines and the thick muscular layer by fine lines. In this and following figures of the same kind the mesenteries are not shown; they of course occupy practically the whole of the enteric cavity after collapse. A section through the aboral end shows conditions similar to those figured in Fig. 2.

In consequence of the infolding about the whole circumference of the cut ends the circumference of the body-wall in the infolded region decreases greatly, although the transverse contraction of the body-wall during the infolding is not marked. It is, therefore, thrown into numerous longitudinal folds and ridges at the edge, and these appear when the piece is viewed from the end as folds and ridges radiating from what remains of the central opening.

Fig. 3[1] shows the oral end of a collapsed piece in which infolding has occurred. The numerous radiating foldings of the body wall are evident. Figs. 4 and 5 show the aboral ends of similar pieces. By this unfolding of the cut edges the openings at the ends of the piece are reduced to slits as is seen from



the figures, and various parts of the circumference of the cut edge are approximated, though actual contact between parts of the cut edge cannot occur everywhere, owing to the irregular wrinkling of the margin as it folds inward. Indeed, since the margin does not contract transversely to any great extent as the infolding occurs, actual contact of all parts of the cut edge is a physical impossibility, as it could occur only by the reduction of the cut margin to a point at the center of the circle formed

[1] In Figs. 3, 4, 5, 6, 7, 8, 9, 10, 12 the longitudinal pigmentation of the body is not shown. The new tissue, where present, is indicated by stippling.

by the body-wall. In most cases, however, as the collapsed, more or less flattened piece lies on the bottom of the jar the infolding edges come into contact along the longer margins as in Figs. 3 and 4, leaving an elongated slit between them. In other cases the closure may occur as shown in Fig. 5. In general the form of the end depends wholly upon physical conditions and especially on the form of the transverse section of the piece after collapse.

The infolding of the cut margins is undoubtedly the result of mechanical conditions, though these conditions may themselves be in part reactive in nature. As Loeb has pointed out, an infolding must occur if the inner portions of the body-wall are under greater longitudinal tension than the outer portions. Such a condition may possibly be produced in the muscles near the cut, the inner layers undergoing greater contraction than the outer, but the elasticity of the fibrillar mesoglœa is probably in part responsible. As will be shown later this infolding produces in many cases conditions from which a return to the normal form is impossible. It can scarcely be regarded, therefore, as an adaptive reaction in the stricter sense. The radiating wrinkles and folds upon the end are due simply to the fact that the cut edges do not contract transversely as they are folded in.

As already noted, the result of the infolding is to close the terminal openings more or less completely. The closure is in no case perfect since between the irregular wrinkles there are always numerous interstices which afford communication between the enteron and the exterior. In most cases, however, these are soon blocked by the tenacious slime secreted by the ectoderm and are also frequently more or less completely filled by portions of the mesenteries or the filaments which happen to extend through them from within.

The Closure of the Ends.

The histological changes about the cut margins have not been fully investigated as yet, but it has been determined that growth of new tissue upon the edges begins soon after the cut is made. If after one or two days the infolded end be opened and carefully spread apart a very thin and delicate whitish membrane of new

tissue will be found extending across parts of the opening. While growth undoubtedly begins on all parts of the cut surface, this membrane becomes distinct earlier at those regions where the cut edges are most closely approximated. Frequently when a piece is opened in the manner described the membrane will be found extending across regions corresponding to certain of the wrinkles about the opening but not yet covering the central area.

This method of formation of the thin membrane closing the end is well shown in a piece cut from a specimen of *C. membranaceus*. In this species the body-wall is so thick and stiff and the diameter of the body so great that in short pieces the infolding of the ends is often not sufficient to close the opening. In Fig. 6 a piece of this kind is shown. The new tissue first became evident along the fold *a*, and a day or two later a thin membrane was spread across this fold (Fig. 7, *a*. The new tissue is stippled). A little later still the fold at *b* (Fig. 7) also showed a thin membrane (Fig. 8), which, however, was afterward ruptured by contractions of the piece due to the stimulation incidental to examination. In Fig. 8 it is seen that the new tissue is gradually spreading over the opening from *a*. In Fig. 9 the opening is nearly closed. Several days later closure was complete. The changes in form of the piece as shown in the figures were the result of stimulation caused by the manipulation necessary for examination and drawing. In *C. solitarius* if the pieces are allowed to remain undisturbed at ordinary summer temperature the openings at the ends are usually completely closed by the thin membrane on the third day after operation. In the piece from *C. membranaceus* above described closure was complete after twenty-seven days. In general this species regenerates much more slowly than *C. solitarius*, but here the closure was exceptionally slow.

The membrane is easily ruptured by the contractions of the piece when strongly stimulated and great care is always necessary in the examination of such pieces to prevent rupture. In consequence of contraction the different parts of the margin change their relative positions or the mass of the mesenteries and filaments exerts pressure from within, thus readily causing rupture.

There is little difference as regards time of closure between the

two ends, though in general the oral end is slightly in advance of the aboral end.

Distension of the Pieces with Water.

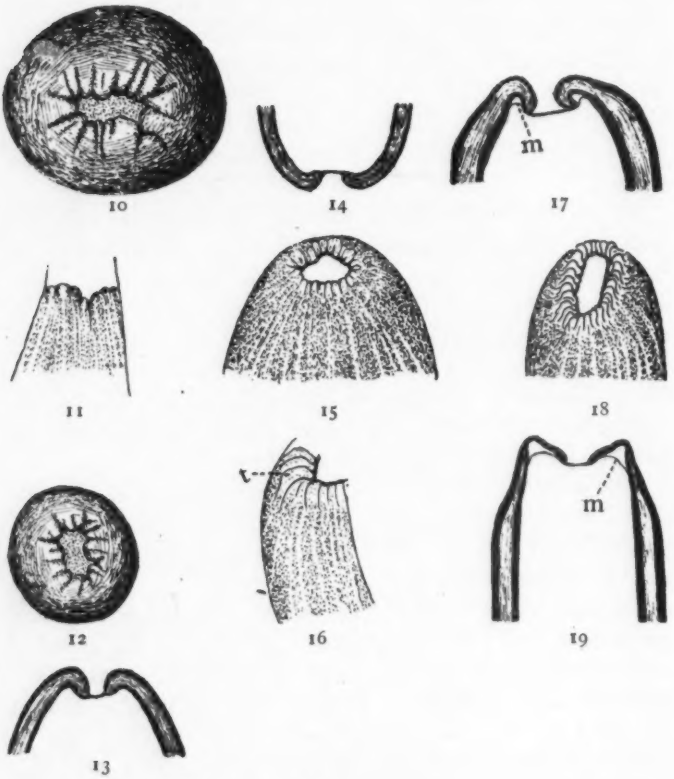
The piece remains completely collapsed during two or three days in summer and five to six days in winter, and then gradually becomes distended. At this time the piece is completely closed at both ends, no mouth or aboral pore being present. It is probable that the accumulation of water in the enteron is the result of diffusion through the walls, and especially through the very thin membranes at the two ends, in consequence of the accumulation of soluble products of metabolism in the closed enteron.

In the course of a day or two the piece becomes well filled with water and attains a degree of distension approaching that of the normal animal, though not as great. In some cases the accumulation of water in the enteron occurs so rapidly that the thin membranes closing the ends are ruptured and collapse occurs again, though usually the increase in thickness and strength of the membrane is sufficient to prevent rupture. The piece is usually well filled with water by the fourth day in summer and usually by the seventh or eighth in winter.

The immediate result of the renewed distension of the piece with water is, of course, the resumption of the cylindrical form; the body wall becomes translucent and is elastic to the touch like that of the normal animal.

The most marked effect of the internal water pressure occurs, however, at the ends of the piece. So long as the piece remains collapsed the thin membrane closing the ends is not visible since the infolded edges of the body-wall are in close contact. As the body becomes distended with water, however, the infolded portions gradually spread apart and a central area covered by the new tissue becomes visible. Very small at first, it gradually increases in size until its diameter is about one third the diameter of the end. Fig. 10 shows the oral end of a piece at about this stage. The area within the folded margin of the old body-wall is covered by the thin membrane of new tissue. In Fig. 12 the aboral end of a similar piece is shown. There is

little difference between the two ends, except that growth of the membrane is more rapid at the oral end. In Fig. 11 [1] a portion of the oral end is shown more highly magnified. In this case the abrupt transition from the pigmented body-wall to the almost colorless new tissues is evident. From this figure it is also seen that the margin of the old body-wall is somewhat crenated by



10 14 17

11 15 18

12 16 19

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fine folds and wrinkles, which, however, are not regular in size and form, and do not represent the early stages of the new tentacles. The slight folds indicate more or less exactly the regions where the mesenteries are attached and the bulging areas

[1] In Figs. 11, 15, 16, 18 the pigmentation of the body-wall is indicated.

between the intermesenterial chambers, these being now filled with water and under pressure. Here and there, however, folds without such significance occur, and moreover some of the chambers are so situated on the infolded margin that they are more widely open and thus expand more in consequence of the pressure than others, hence the irregularity in form and size of these crenations.

In Figs. 13 and 14 are shown respectively the oral and aboral ends of the body-wall at the stage where the infolded portions begin to separate. The thin membrane closing the end is shown as a black line. It consists, of course, of ectoderm and entoderm, but the muscular layer does not extend into it.

The Formation of the Marginal Tentacles and Disc.

Within the first day or two following the closure of the ends and the distension of the piece with water the changes leading to the formation of the characteristic organs of the oral end begin. In pieces cut from the middle region of the body the full number of mesenteries is not present, since some end anterior to this region. Regeneration of mesenteries occurs, though the number of mesenteries in a regenerated oral end from the middle region of the body is somewhat less than the number originally present at the oral end of the individual from which the piece is taken. This point will be considered at another time. It is sufficient for the present purpose to say that the whole oral end of the piece becomes divided into intermesenterial chambers, in the manner characteristic of the species, by the regeneration of new mesenteries, at first very short, between the longer mesenteries which are present in the piece. Attention was called above to the crenation of the infolded margin in correspondence with the position of the mesenteries (Fig. 11).

The first marked change following the closure of the end is the appearance of a slight ridge on the infolded margin of the old body-wall as shown in Fig. 15. The ridge is wholly confined to the tissue of the original body-wall, the thin membrane which closes the end playing no part in its formation. The crenations become more distinct and extend in many cases from the margin of the old body-wall over the ridge, as the regeneration of the

mesenteries beneath advances. In Fig. 15 the ridge is shown as slightly lighter in color than the rest of the body. The pigmentation is beginning to disappear. Most of the stripes can still be followed over the ridge to the margin of the old tissue, but upon the ridge they are fainter than before. Fig. 16 shows a portion of the end at a slightly later stage, more highly magnified. Here the lighter color of the ridge is more distinct. While the body in general retains its brown color the ridge becomes light yellowish and its pigment disappears completely in the course of a day or two.

This change in pigmentation indicates that some alteration in the tissues is occurring, and the nature of the alteration becomes evident when a longitudinal section through the end (Fig. 17) is examined. This figure shows that the thickness of the body-wall and especially of the muscular layer is decreasing considerably in the region corresponding to the ridge. This decrease is shared to a certain extent by the ectoderm and entoderm as the figure indicates. The new regenerating mesenteries are minute folds in the infolded region, ending free aborally (*m*, Fig. 17).

This ridge in which loss of pigmentation and reduction in thickness of the body-wall are taking place may be designated as the *marginal tentacular ridge*, since it is from this that the marginal tentacles arise; indeed the reduction in thickness of the body-wall and the division of the ridge into areas corresponding to the intermesenterial chambers are the preliminaries of tentacle formation.

The marginal tentacles do not arise from the cut edge of the body-wall itself but a short distance away from it, viz., at the highest point of the ridge (*t*, Fig. 16), *i. e.*, entirely within that portion which was originally part of the body-wall and not in the new tissue which closes the end.

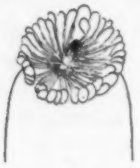
Fig. 18 shows the oral end of a piece about a day later than the stage shown in Figs. 15 and 16. Here the new marginal tentacles are distinct and are evidently increasing in length. The pigment has disappeared completely from the tentacular ridge which is now whitish in color and distinctly translucent. Some of the tentacle buds are slightly broader than others owing to the fact that in the infolded condition of the margin some inter-

mesenterial chambers were compressed and others stretched according to their position on the folds. There is, however, no marked difference in the length of the new tentacles on the different sides of the body, those in the region of the directives being no more advanced than those in the growing region opposite. From this figure it is very evident that the marginal tentacles arise from the highest, *i. e.*, the most oral point of the tentacular ridge. Moreover they arise in a single circle or row, although in the normal animal they occur in about three concentric circles.

A longitudinal section of the body-wall at this stage is shown in Fig. 19. A comparison with Fig. 18 shows marked changes. The most conspicuous of these is the continued reduction in thickness of the body-wall upon the ridge. The muscular layer has almost or quite disappeared in this region and also between it and the new tissue occupying the central region of the end, and is reduced considerably in thickness for some distance aboral to the ridge. At this stage then the whole oral end is closed by a thin, unpigmented, translucent membrane consisting of ectoderm and entoderm, but without a distinct muscular layer. The central part of this membrane resulted from the growth of new tissue at the cut edge, while the more distal portions forming the tentacular ridge have arisen by the transformation of a part of the old body-wall into tissue capable of a large amount of new growth, and of differentiation into new structures. In other words, the body-wall in this region has changed from its differentiated condition to what is commonly called the embryonic condition. The histological features of this change are of great interest, but will be described at another time.

The marginal tentacles now grow rapidly, and in another day (six days after the operation in summer) the oral end presents the appearance shown in Fig. 20. Several changes of importance have occurred since the stage of Fig. 18: the disc is greatly expanded, the marginal tentacles are much longer, the distinction between the tissue of the old body-wall and the thin membrane closing the end has disappeared completely, and finally the mouth is beginning to appear as an opening between the center and the periphery of the disc in the directive radius. The disc is

marked with radiating lines, each of which terminates distally between the bases of two tentacles; those lines are in reality grooves and mark the lines of attachment of the mesenteries to the aboral surface of the disc. It will be seen that a small area



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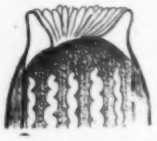

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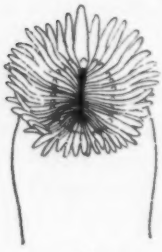

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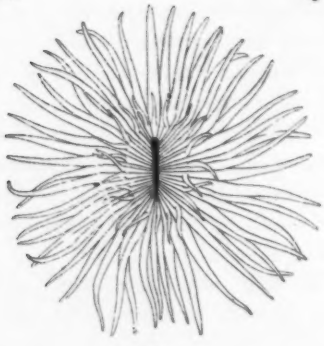

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in the center of the disc, indicated in the figure by stippling, is free from these lines; this represents that portion of the thin membrane beneath which regeneration of the mesenteries has not yet occurred. In the directive radius is situated a small opening, the new mouth, which gradually elongates in the directive plane.

The directive tentacle, which corresponds to the chamber between the two directive mesenteries, is slightly thicker than the other tentacles in consequence of the fact that the directive mesenteries are somewhat farther apart than other mesenteries. As regards the arrangement of the marginal tentacles it will be seen that they are no longer in a single row, but some appear as if they were being crowded out of the row, owing to lack of space. In all probability that is what is occurring. As the tentacles increase in size there is not sufficient space for them in a single row upon the margin and some are pushed out, probably in most cases peripherally.

Fig. 21 is a schematic figure of one half the body after longitudinal section in the directive plane, the directive tentacle being on the left of the figure. The stage of regeneration is about the same as that of Fig. 20. As compared with the earlier stages (*e. g.*, Fig. 19) several points of difference are to be noted: the marginal tentacles are longer, the difference in thickness between the reduced body wall of the tentacular region and the thin new tissue across the disc has completely disappeared; the reduction and disappearance of the muscular layer extends further aborally than before; the regeneration of the mesenteries has advanced; and finally there is a minute mouth, which, as was evident from Fig. 20, is not centrally placed, but lies near the base of the directive tentacle.

One or two points of importance as regards the regeneration of the mesenteries may be noted. In the normal animal a slight furrow, which appears as a faint longitudinal line on the surface of the entoderm, extends aborally from the aboral end of each mesentery. In a piece cut from the middle region many of the mesenteries lie wholly oral to the cut and so are not present in the piece, but most of the furrows, extending aborally, are visible in the piece. The mesenteries regenerate along these furrows. Whether regeneration of a particular mesentery aboral to the end of the furrow representing that mesentery is possible has not yet been determined. The point to which it is desired to call attention here is that the mesenterial regions are determined for some distance posterior to each of the mesenteries themselves.

Those mesenteries which extend into the piece undergo regressive changes, losing their thickened margins and filaments at the oral end, and become united with the new œsophagus.

The Appearance of the Labial Tentacles and the Later Stages of Oral Regeneration.

The marginal tentacles continue to increase rapidly in length and the œsophagus extends further across the disc from the directive side and also becomes deeper.

Fig. 22 is drawn from a stage three days later than Fig. 20 (nine days after the operation). Comparison of this figure with Fig. 20 shows at once the increased diameter of the disc, the greater length of the tentacles, and the marked change in the size and shape of the mouth opening. The tentacles in Fig. 22 are still of about equal size and length, except the directive tentacle, which is somewhat thicker and longer than the others. They still retain, to a large extent, the arrangement in a single row, though here and there a few have been forced out of line.

Upon the disc and forming a circle about the mouth appear the earliest stages of the labial tentacles. They are at this time mere buds, less than one half millimeter in length. All appear nearly simultaneously and develop with equal rapidity. As noted above, they are fewer in number than the marginal tentacles, some of the intermesenterial chambers possessing none.

A view of half the oral end at the stage of Fig. 22 after longitudinal section in the directive plane is shown in the schematic Fig. 23. In this case the plane of section passed through one of two small tentacles in the growing region opposite the directive tentacle; the section of this tentacle (on the right of the figure) is thus considerably smaller than that of the directive tentacle opposite. Comparison of Figs. 21 and 23 shows the changes which have occurred during the three days elapsing between the two stages. The œsophageal invagination is much deeper, the opening to the enteron is larger and the area of growing tissue, including the reduced body-wall, is much greater.

From this time on the course of regeneration consists in the gradual increase in size and the pigmentation of the regenerated parts in the manner characteristic of the species.

The problem of "morphallaxis," *i. e.*, the changes in the proportions of regenerating pieces leading to the more or less complete reëstablishment of the "normal" form will be considered elsewhere.

Fig. 24 shows a regenerated disc and tentacles at a later stage; in form and general arrangement of parts it is not distinguishable from the normal animal. The marginal tentacles have not yet fully attained their final arrangement; at present they are in two fairly well marked rows or circles. During the still later stages, however, as further increase in size occurs, the bases of some are forced still farther peripherally and so the characteristic arrangement of tentacles is finally acquired. The pigmentation of the marginal tentacles with dark transverse bands, which appears at this stage or earlier, is not shown in the figure.

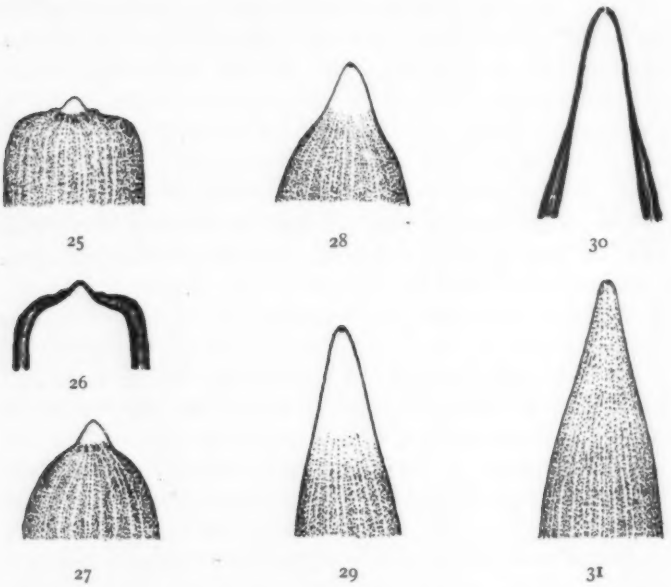
The Differentiation of the Aboral End.

The infolding of the body-wall and the closure of the aboral end of a piece by a thin membrane have already been described. It remains to describe the formation of the characteristic aboral end. The course of regeneration here is much simpler than at the oral end.

The first marked change from the condition shown in Fig. 14 consists in the protrusion in conical form of the thin membrane closing the end (Fig. 25). About the margin of this new tissue the slightly wrinkled margins of the old body-wall are still clearly marked. In Fig. 26 a longitudinal section of the aboral end at this stage is shown. The absence of the aboral pore is to be noted. This new outgrowth at the aboral end does not become well-marked at once after closure, but only after the piece is well filled with water and the regeneration is advanced at the oral end, *i. e.*, it is much slower than oral regeneration.

In Fig. 27 the aboral outgrowth is seen at a somewhat more advanced stage. The wrinkles and folds upon the margin of the old tissue are gradually disappearing as this stretches and undergoes remoulding. A few days later the wrinkles have disappeared and there is no sharp distinction between the old body-wall and the new tissue at the time of union. Fig. 28 shows the end at this stage; and it is evident that the margins of the

old body-wall are becoming involved in the regenerative changes in the same manner as at the oral end, for the pigment stripes are gradually fading out in the region which was before infolded. Fig. 29 shows a still later stage in which the gradual fading of the pigment-stripes is clearly seen. The significance of this loss pigment is made clear by Fig. 30, a longitudinal section of the aboral end at this stage. Here it is seen that a reduction of the muscular layer is occurring, *i. e.*, the old body-wall is becoming



involved in the regulative changes for a short distance oral to the cut end: In other words the new aboral end is formed not merely from the new tissue which closes the end soon after operation, but, as in the regeneration of the oral end, in part from tissue derived from the margins of the body-wall near to the cut surface, by reduction of the muscular layer and growth of the ectoderm and entoderm. Thus the distinction between "old tissue" and "new tissue," at first well-marked, gradually disappears in this region.

Fig. 31 shows a still later stage in which the new tissue is becoming pigmented. The appearance of the pigment corresponds in time with the differentiation of the muscular layer, and I am inclined to believe that in *Cerianthus* as in various other forms, the pigmentation of the body is closely connected with the presence and arrangement of the muscular layer.

The course of regeneration described in the present paper is characteristic of pieces cut from the middle half of the body. In following papers the regeneration of pieces from various regions will be compared, and experiments determining some of the factors concerned in regeneration will be described.

Summary.

1. In cylindrical pieces of *Cerianthus* obtained by two transverse cuts collapse occurs at once and the cut ends begin to roll inward soon after section, finally coming into contact and closing the opening more or less completely. Since little or no transverse contraction of the infolded margins occurs they are thrown into numerous radiating folds and wrinkles.

2. Within two to three days after section a thin membrane formed by the growth of new tissue from the cut surfaces closes the two ends completely. The piece now becomes gradually distended with water, probably owing to the accumulation of metabolic products in the enteron and consequent diffusion of water into this closed cavity. As distension proceeds the infolded margins of the body-wall at the two ends are forced apart by internal pressure and the area occupied by the thin membrane increases.

3. The first step in the regeneration of tentacles is the formation of a slight ridge, the marginal tentacular ridge, on the oral end. This ridge is formed wholly within the tissue of the old body-wall, its formation being accompanied by reduction and disappearance of the muscular layer, disappearance of the pigment and great reduction in thickness. The marginal tentacles first appear as slight upgrowths from the highest — most oral — point of the ridge, one tentacle corresponding to each intermesenterial chamber. The position of the mesenteries is indicated externally on the tentacular ridge by slight furrows which separate the regenerating tentacles from each other.

4. The regenerating marginal tentacles appear at first in a single circle and all usually regenerate with nearly equal rapidity, except in some cases the youngest pair in the growing region. The directive tentacle is usually slightly thicker than the others since the directive mesenteries are somewhat farther apart than the other mesenteries. Rapid increase in length occurs in the marginal tentacles, and the arrangement in about three circles or rows is gradually attained in consequence of the fact that there is not sufficient space on the margin of the disc for all of the tentacles in a single row; some are forced peripherally by the mutual pressure exerted.

5. As the tentacles grow the disc expands and the distinction between the thin membrane of new tissue which first closed the end and the old body-wall with which it was connected disappears completely in consequence of the complete disappearance of the muscular layer, the reduction in thickness, and the loss of pigment in the body-wall of the oral end.

6. The mouth appears after the marginal tentacles are well established near the base of the directive tentacle, gradually extending along the directive plane across the center of the disc until it is symmetrical. The part of the mouth first regenerated is the region of the siphonoglyph.

7. The labial tentacles do not appear until the marginal tentacles have attained a length of several millimeters. Each tentacle appears as a distinct bud over an intermesenterial chamber, but some intermesenterial chambers are without labial tentacles.

8. After the aboral end is closed by the new tissue this slowly acquires a conical form, protruding from within the wrinkled margin of the old body-wall. The wrinkles on the latter gradually disappear and the pigmentation slowly fades out for a short distance oral to the cut end, this change being connected with reduction and disappearance of the muscular layer as this region of the body-wall becomes involved in processes of growth and redifferentiation in the same manner as the oral end. The aboral end grows out into an elongated conical form at the end of which the aboral pore appears. As the new muscles differentiate in this region pigment stripes begin to appear.

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,
July, 1903.

THE EYES OF THE BLIND VERTEBRATES OF NORTH AMERICA. VI.[1] THE EYES OF TYPHLOPS LUMBRICALIS (LINNÆUS), A BLIND SNAKE FROM CUBA.[2]

EFFA FUNK MUHSE.

Typhlops lumbricalis,[3] a blind snake, is generally distributed in the West Indies and Guiana. The specimens examined were obtained by Dr. C. H. Eigenmann in the neighborhood of Cañas, Province Pinar del Rio, Cuba. It is a burrowing form, that lives just beneath the surface, being thrown out even by the plow.

The snakes were first placed in formalin and after a few days were changed into alcohol. Only one young specimen was obtained, and it was preserved in Zenker's fluid. For decalcification, the heads of some were placed for at least three days in ten per cent. nitric acid and others in Perenyi's fluid from one to two weeks. One series was stained by the iron hæmatoxylin process, the others with hæmalum and eosin. It was very difficult to obtain satisfactory sections and especially complete series from the specimens, since no method was found to decalcify properly and to get the integument in condition for sectioning.

The lengths of the individuals examined were 10, 20, 21 and 21.5 cm. The color is brown above, on the ventral side it is yellowish-white. The body is covered with scales of uniform size, while those of the head are somewhat larger. The surface of the entire body is very smooth and shining and rather hard. The tail, which is about one twentieth of the body's length, ends in a short, sharp spine. The mouth is small and lies on the ventral side some distance back from the tip of the snout.

I. Normal Eyes of Snakes.

Snakes differ from other animals in having the edges of the two eyelids entirely grown together. A disk-shaped, conjunctival

[1] Contributions from the Zoölogical Laboratory of Indiana University under the direction of C. H. Eigenmann.

[2] The blind vertebrates of Cuba are rated with those of North America.

[3] Boulenger, G. A., "Catalogue of the Snakes in the British Museum," 1893.

sac is thus formed and the layers over the eye between this sac and the exterior form the "brille."

Six weakly developed muscles are present. The four straight ones arise in the neighborhood of the foramen opticus, while the two oblique ones arise from the surface of the prefrontal which is turned toward the eye socket.

Closely connected with the eye is a gland, Harder's, whose function is doubtful. Leading from this gland is a single duct, which either empties into the duct from Jacobson's gland or directly into the mouth cavity. The secretions of the gland are thus not functional in connection with the eye.

The sclera consists of closely woven fibers. Ciliary muscles are not found, but next to the iris is a great bundle of equatorial muscle fibers running obliquely, which seem to be a continuation of the iris musculature. The ciliary processes are weakly developed.

The retina consists of the usual layers. The nerve fiber layer is very thin (.003–.004 mm.).

The ganglion cell layer consists of a single, rarely two layers of small cells, each with a very large nucleus (.012–.013 mm.).

The inner reticular layer contains, at apparently regular intervals, elongated, oval nuclei (.042–.045 mm.).

The inner nuclear layer consists of two kinds of cells (.052–.054 mm.).

The outer reticular layer is very thin (.004–.005 mm.).

The sensory epithelium consists of the outer nuclear layer and the cone layer which is made up of single and twin cones. There are no rods. A single cone consists of two sections, an outer extremely small section, 5–6 microns in length and an inner much larger section, almost completely filled with a larger, pear-shaped, strongly refractive body, the ellipsoid, 14–16 microns in length and 8–9 microns across its widest part, which is turned toward the limiting membrane. The twin cone consists of two parts, one similar to a simple cone, the other cylindrical and very slender, its structure being otherwise like that of a simple cone. It is probable that the two parts of the twin cone are connected with but one nucleus. The nuclei of the cones vary greatly in form and leading from these into the inner layers of the retina are relatively very large fibers or processes.

Passing between the limiting membranes are the radial supporting Müllerian fibers.

II. The Eye of *Typhlops vermicularis.*

The work thus far on blind snakes has been done by Kohl on *Typhlops vermicularis*, a species found in Greece and the southwestern part of Asia, and on *Typhlops braminus*, a species found in the islands of the Indian Ocean and in Africa south of the equator, accounts of which are given in his "Rudimentäre Wirbelthieraugen."[1]

He found that in depth the eye of *Typhlops vermicularis* is equal to about one sixth that of *Tropidonotus.*

The brille is thicker in *Typhlops* than in *Tropidonotus* and, compared with the axial diameter of the respective eyes, is seven times thicker. In *Typhlops* the brille is equal in thickness to about one half that of the ordinary skin of the head. In *Tropidonotus* it is equal to one fourth.

The cornea of *Typhlops* measures .0052 mm., and compared with the relative sizes of the eyes is equal to about one half that of *Tropidonotus*, which measures .064 mm.

The conjunctiva is thickened at the edge of the disc-shaped sac and consists here of gland cells, the fornix conjunctiva.

The supporting membranes of the eyeball, choroid and sclera are relatively equal to about one half those of *Tropidonotus.*

Harder's gland in *Typhlops* is many times larger than the eyeball.

The six muscles are present.

The lens is elliptical, while that of *Tropidonotus* is almost globular. The ratio of the lens volume of *Typhlops* to the eye volume is 1 : 14.04, while in *Tropidonotus* it is 1 : 3.6. The lens epithelium of the former is relatively six times greater than that of *Tropidonotus.*

The retina at the back of the eye of *Typhlops*, and the retina of *Tropidonotus* bear the actual ratio of 8 : 13, while compared with the eye axis in each case the *Typhlops*-retina is four times greater. The fovea centralis and area are absent.

[1] Kohl, Dr. C. "Rudimentäre Wirbelthieraugen," Erster Thiel, Heft. 13, Bibliotheca Zoologica. Verlag von Theodor Fischer, 1892, Cassel.

The fiber layer has its greatest thickness near the exit of the nerve and gradually becomes thinner until, near the iris, scarcely a fiber is found.

The globular ganglion cells are arranged in a single layer except occasionally for short distances, when they lie in a double row.

The inner nuclear layer seems to be subdivided into four layers.

There are no twin cones. Each cone consists of a cone cell, stalk, middle and end members. The cone nuclei lie in two series, but the stalks vary in length so that the distal ends of the cone members reach nearly the same level.

III. The Eye of *Typhlops lumbricalis.*

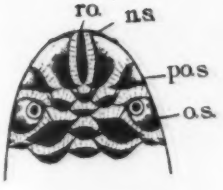
The eye shows through the large ocular scale, which entirely covers it. It appears as a black spot surrounded by an unpigmented circle. The preocular, also a large scale, overlaps the ocular and reaches just to the edge of the eye (Figs. 1 and 2).

General Account of the Eye.

Compared with one of the garter snakes and in proportion to the size of the head, the eye of *Typhlops lumbricalis* is located further from the surface and occupies far less space, while Harder's gland, associated with the eye in both, is relatively much larger in *Typhlops.* In a specimen of *Typhlops lumbricalis* 21 cm. in length, the eye measured .306 mm. in width, and .387 mm. in depth. The greatest width of the gland of the same was .711 mm. and the length was 1.067 mm. The gland completely surrounds the eye up to the edges of the conjunctival sac (Figs. 3 and 4). In proportion to the size of the eyes, the gland of a garter snake is much smaller than that of *Typhlops lumbricalis,* but compared with *Rhineura floridana*[1] the gland of *Typhlops lumbricalis* is but little more than half as large.

The eye is covered by layers of epidermis and dermis, that differ from these same layers on neighboring parts by being thinner, more compact and free from pigment and glands. The ocular scale, however, which covers the eye region, does not differ in thickness from the other scales of the head (Fig. 3).

[1] Eigenmann, C. A., "The Eyes of *Rhineura floridana,*" *Proceedings of the Washington Academy of Sciences,* Vol. IV., pp. 533–548, Sept. 30, 1902.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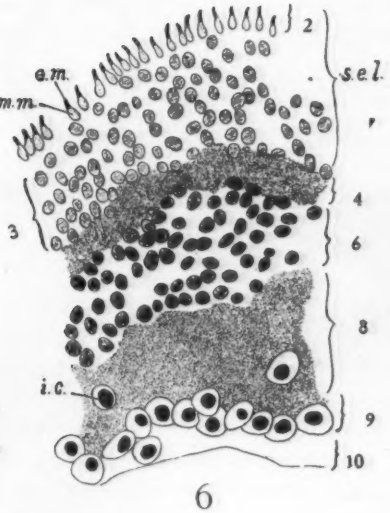
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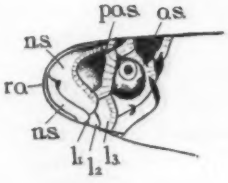
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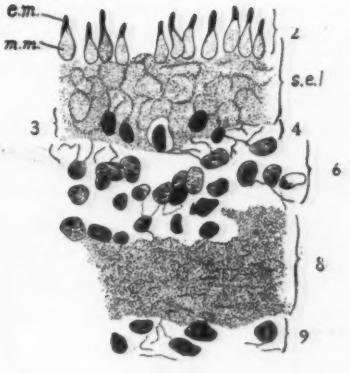
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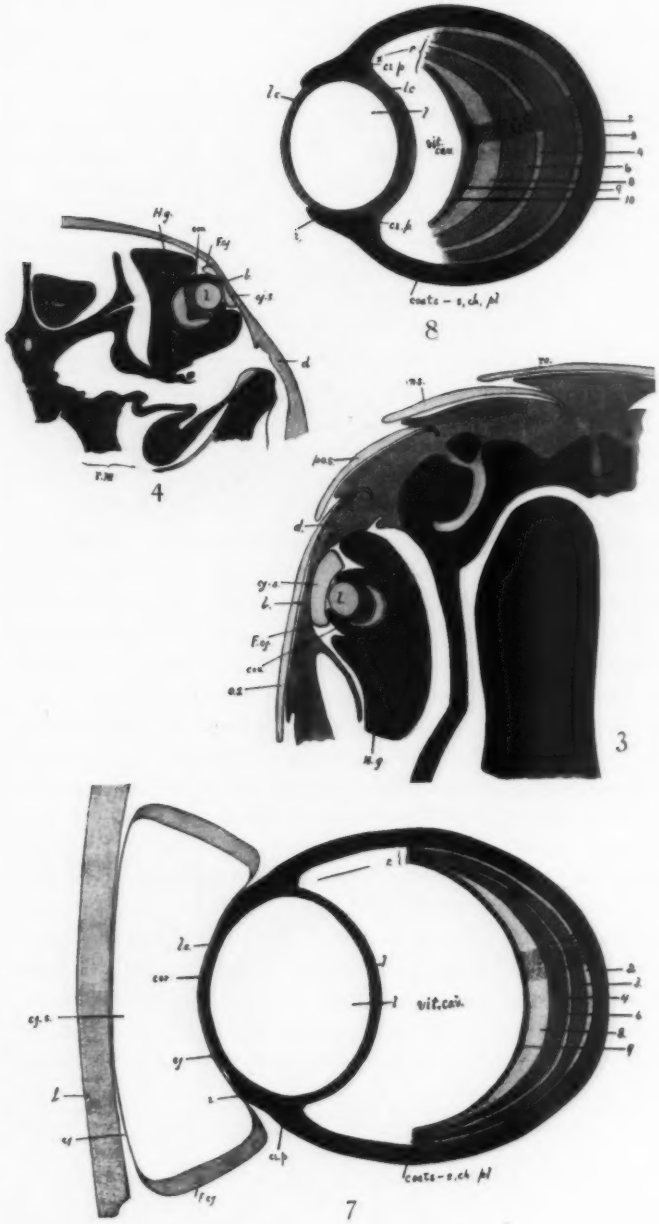
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A conjunctival sac is present with a diameter at least as great as the greatest width of the eye bulb. The conjunctiva, which forms this sac, is very thin over the cornea and next to the brille where it measures .003 mm. At the edge of the sac, it is differentiated into glands, the fornix conjunctiva, and measures .016 mm (Figs. 3 and 4).

In horizontal section, the eye axis is seen to be turned forward about 30° away from a line at right angle to the horizontal axis of the body.

Eye muscles are present, but from the sections used, the exact number could not be determined.

Minute Anatomy of Eye.

Choroid and Sclera.—The dense pigmentation makes it impossible to distinguish between the different coats at every point. Beyond the retina with its pigment layer is an open vascular space and this is followed by another dark layer, the two together representing the choroid. The choroidal pigmentary layer seems to consist of long fibers circularly arranged. The sclera can be followed by starting with the outer covering of the optic nerve and tracing its continuation about the eye.

Iris and Ciliary Processes. — Here again the pigmentation makes it difficult to determine the structure. Both iris and ciliary processes are present, for the black layer extends over the anterior surface of the lens, leaving a pupil equal in diameter to about one fourth of the circumference of the lens. At points near the equator of the lens this dark layer is enlarged into the ciliary processes and in connection with the capsule helps to hold the lens in place (Figs. 3 and 4).

Cornea. — This structure is present and can be traced to the region of the ciliary processes.

Lens. — A large lens is present, its depth being equal to about two fifths of the eye depth. From the sections little could be determined about its structure. A well-developed capsule surrounds it (Fig. 7).

Retina. — The same layers are present that are found in snakes in general, but the comparative thickness of the various layers is different. In the garter snakes, for instance, the retina is of a

uniformly even thickness even to the ciliary process, a single layer of cells continues on over the surface of the processes and iris, but in *Typhlops lumbricalis* the retina at the back of the eye is very thick and gradually becomes thinner till it ends a short distance from the ciliary processes (Fig. 7). At this point the arrangement could not be definitely determined in the sections. At the back the retina, exclusive of the pigment layer, measures .0725 mm.

Ends of fibers were seen projecting inward from the ganglion cell layer, but no definite fiber layer could be distinguished (10 in Fig. 5).

The ganglion cell layer (9 in the figures) consists of a single row of large nucleated cells, somewhat irregularly arranged (.008 mm.).

The inner reticular layer (8) consists of a mass of fibers interwoven in a close network. This layer measures, at the back of the eye, .015 mm.

The inner nuclear layer (6) consists of at least three layers of cells, loosely arranged. The course of some of the fibers can be followed among these cells. This layer measures .016 mm.

The outer reticular layer (4) is very thin and consists of a few fibers so arranged as to leave a great number of spaces between the two nuclear layers. The distance between the nuclear layers is about .005 mm.

The sensory epithelium shows two distinct parts, an inner layer of nuclei (3) and an outer row of cones (2). In the sections these two were so separated that a loose tissue was visible, consisting probably of the limiting membrane and ends of the Müllerian fibers. The outer nuclear layer in the adult consists of a single row of nuclei, with a mass of quite homogeneous material about them. This part of the sensory epithelium measures .018 mm. The cones are pear-shaped bodies with the smaller end pointing outward, and at intervals of every four or five a shorter one occurs. Each element is differentiated into two parts. By the iron hæmatoxylin process of staining, the outer small end is densely stained, while the body of the element is a light granular mass (Fig. 5).

The pigment layer (1) is a continuous layer of even thickness, similar in every respect to that of the garter snake.

One young specimen, 10 cm. in length, was examined. The eye as a whole, as well as the lens, is nearly spherical. The eye measures in width .290 mm. and .322 mm. in depth. All parts are so developed that the vitreous cavity is relatively much smaller than that of the adult. The coats are thicker, the ciliary processes better developed, the lens capsule thicker, and the retina at the back actually measures one and two thirds the depth of the adult retina. The elements of each layer are much more numerous than in the adult, and they are packed much more closely together (Fig. 6). The ganglion nuclei are apparently arranged one against the other. In the inner reticular layer occur the "interpolated cells." These were not found in the sections of the adult eye that were examined. The cells of the inner nuclear layer are smaller and arranged in five or six rows. There is a well-developed outer reticular layer similar in its make-up to the inner reticular. Instead of a single row of cone nuclei with its surrounding homogeneous mass, as in the adult, this layer in the young consists of five or six rows of small, closely arranged cells. The cones likewise are smaller and more numerous (Fig. 6).

COMPARATIVE MEASUREMENTS OF RETINAL LAYERS IN MM.

	Fiber Layer.	Ganglion Cell Layer.	Inner Reticular Layer.	Inner Nuclear Layer.	Outer Reticular Layer.	Sensory Epithelium.	Total Depth.
Tropidonotus natrix.	.003	.012	.042	.052	.004	.0196	.1331
Typhlops vermicularis.	.0018	.0081	.0155	.0221	.0022	.0324	.0821
Typhlops lumbricalis (adult).		.008	.015	.016	.005	.030	.0725
Typhlops lumbricalis (young 10 cm.).	.005	.010	.024	.032	.008	.040	.1206

RELATIVE PROPORTIONS OF EYE PARTS.

		Tropidonotus natrix.	*Typhlops vermicularis.*	*Typhlops lumbricalis* (adult).
Eye depth.		2.5541 mm.	.4399 mm.	.4032 mm.
Brille :	Eye axis ::	1 : 77.4	1 : 10.77	1 : 12.5
Cornia :	Eye axis ::	1 : 39.9	1 : 84.6	1 : 85
Lens depth :	Eye axis ::	1 : 1.56	1 : 3.03	1 : 2.5
Coats :	Eye axis ::	1 : 21.63	1 : 38.58	1 : 25.4
Retina at back :	Eye axis ::	1 : 19.19	1 : 5.36	1 : 5.5

Explanation of Figures.

Figs. 1 and 2 are from entire specimens. All figures except 1 and 2 are from sections. Figs. 7 and 8 are diagrams.

Explanation of Notations Used.

b. Brille.
ch. Choroid.
ci.p. Ciliary processes.
cj. Conjunctiva.
cj.s. Conjunctival sac.
cor. Cornea.
cov. Coverings of eye.
d. Dermis.
e.m. End member of cone.
F.cj. Fornix conjunctiva.
Hg. Harder's gland.
i. Iris.
i.c. Interpolated cells.
l. Lens.
l_1. First labial scale.
l_2. Second labial scale.
l_3. Third " " .
l.c. Lens capsule.
m.m. Middle member of cone.
n.s. Nasal scale.
o.c. Ocular scale.
p.l. Pigment layer.
po.s. Preocular scale.
r. Retina.
ro. Rostral.
r.m. Roof of mouth.
s. Sclera.
s.e.l. Sensory epithelium layer.
vit.cav. Vitreous cavity.

1. Pigment layer.
2. Cones.
3. Outer nuclear layer.
4. Outer reticular layer.
6. Inner nuclear layer.
8. Inner reticular layer.
9. Ganglion cell layer.
10. Fiber layer.

Fig. 1. Dorsal view of head of a specimen 21 cm. long.

Fig. 2. Lateral view of head of same specimen.

Fig. 3. Horizontal section of a specimen 20 cm. long, $\frac{2}{3}$-objective, 2-inch eye piece, camera lucida.

Fig. 4. Transverse section of a specimen 21 cm. long, $\frac{2}{3}$-objective, 2-inch eye piece, camera lucida. (Scales not shown.)

Fig. 5. Section of retina of an adult specimen 21 cm. long, $\frac{1}{12}$-objective, 1-inch eye piece, camera lucida.

Fig. 6. Section of retina of young specimen, 10 cm. long, $\frac{1}{12}$-objective, 1-inch eye piece, camera lucida.

Fig. 7. Diagrams of eye of adult.

Fig. 8. Diagram of eye of young.

(The region *x–r* in the sections could not be made out and is consequently left blank in the diagram.)

SOME EXPERIMENTS IN FEEDING LIZARDS WITH PROTECTIVELY COLORED INSECTS.[1]

ANNIE H. PRITCHETT.

During the past year, from October to May inclusive, I have been experimenting with insects that possess protective, mimetic and warning colors or that have some disagreeable characteristics which in a measure are supposed to prevent their being devoured by insect-eating animals. For this purpose several species of lizards found in the vicinity of Austin, Texas, have been kept in separate, convenient cages and fed with the various insects. Some interesting observations on the habits of the lizards were made incidentally and these are also noted in the following paper.

The species of lizards used for the experiments are the following: *Gerrhonotus infernalis* Baird, *Chrotaphytus collaris* Say, *Sceloporus floridanus* Baird, *Holbrookia texana* Troschel, *Cnemidophorus sexlineatus* Linn., *Phrynosoma cornutum* Harl., and an undetermined species of *Eumeces*.

Experiments with Sceloporus floridanus.

Lepidoptera.

Anosia plexippus Linn. This species is conspicuously colored in light brown with black and white markings. It is also said to have a disagreeable taste and is the supposed model of the mimic *Basilarchia disippus*. Specimens were introduced October 31, November 6, April 2 (two), April 4, April 6. Each time the butterfly was caught by the wing, or by the wings if folded, held for a few moments and then eaten slowly. It was not torn to pieces but held by part of the wings and swallowed gradually, the lizard often pausing a moment to rest.

Papilio (*Laërtias*) *philenor* Linn. Formerly this was included in the genus *Papilio* but has been separated because of characteristic differences, important among which is the supposition that it is an especially protected form because its larva feeds on *Aris-*

[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 52.

tolochia, a poisonous plant of disagreeable taste. On October 30, March 27, March 30 (two), March 31, April 1 (three), April 4, April 16, April 23, May 4 (four), May 6 (two), butterflies were introduced into the cage and quickly eaten by the lizards with evident relish. On May 6 one of the specimens was badly mutilated and the lizards were not induced to take it for more than an hour.

Pieris occidentalis Reakirt. October 29, April 20 (three).

Pieris protodice Boisd-Lec. April 23, May 1 (four). These forms, white with black markings, were readily eaten.

Colias eurytheme Boisd. November 8 (two), March 31 (two), April 9, April 20 (seven), April 23 (five), May 1 (two). All quickly eaten.

Colias ariadne Edwards. April 16 (two).

Colias scuddere Reakirt. April 20, May 1 (two). These species are of striking yellow or orange marked with black, a typical warning combination, yet all were eaten eagerly.

Pyrrhanæa andria Scudder. This form is admirably protected by having the under side of the wings an exact imitation of a dead leaf. The wings are held folded closely together when the butterfly is at rest, and it remains motionless in this position for a great length of time. It is one of the most perfect instances of protective resemblance that I have obtained. Specimens were introduced November 14, April 22 (two, ♂ and ♀) and April 27. On April 22 the butterflies were not noticed at first. Several times they were offered to the lizards; the male was taken in about five minutes and the female ten minutes later. On April 27 the butterfly was seized by the wings several times, then dropped again. It remained motionless unless I moved it and the lizard would then seize it again. Finally it was abandoned, but it had disappeared the next day and probably had been eaten at last.

Pyrameis atalanta Linn. November 29. This is a conspicuous form, of black, brown, red and white. The lizards ate it eagerly.

Pyrameis huntera Fabr., a similar form but having large eye-spots underneath the wings. It was eaten May 1.

Grapta interrogationis Fabr. April 1. This species also has the under side of the wings in imitation of a dead leaf, and is

very difficult to detect when at rest. It is in the habit of remaining motionless for a long while. The specimen introduced was at once eaten.

Papilio cresphontes Cramer. One specimen was introduced April 23 and four lizards at once seized the outspread wings. They showed no preference for the body but ate the wings first, as is usually the case. On May 7 the wings of the specimen introduced were almost entirely eaten when the lizard happened to drop it. It remained quiet, and the lizard would only take it again after I had made the butterfly move several times.[1]

Deilephila lineata. May 5. Two of these Sphingid moths were introduced and seized at once. They fluttered continuously and thus frustrated the attempts of several other lizards that were trying to participate. One moth was held by the head, the other by the wing for quite a while, till they ceased fluttering, and were then eaten.

Species unknown. May 4. This small moth is of black and orange, the typical warning coloration. It was eaten at once without any symptoms of dislike being shown.

HEMIPTERA.

Lygæid. May 5. Just after the above-mentioned moth was eaten four of these bugs were introduced. They are of the typical black-and-red or orange warning colors and have a very disagreeable odor. The same lizard that ate the moth at once seized a bug, chewed it a moment and spit it out, then licked his mouth for some time as if to remove the bad taste. Another lizard examined a second bug but made no attempt to take it. One bug was eaten later by the third lizard and the other two were gone next morning. May 13 a bug was introduced, seized at once and then rejected as before. It is evidently quite unpalatable.

Brachymena myops. Three were introduced November 8, but were never noticed by the lizards. The bug is gray in color,

[1] A glass jar containing live butterflies was placed on a chair about two and one half or three feet from the cage of *Sceloporus*. A large male lizard immediately climbed up the side of the cage, eyed the butterflies eagerly and seemed quite excited. This happened a few days later with several of the lizards. When the insects were introduced they were seized and eaten at once, several lizards quarreling over a desirable specimen and sharing it among themselves.

quite similar to the bark of trees that it frequents, and possesses a very unpleasant odor.

Fulgorid. Introduced November 5, November 6. This lantern fly is almost impossible to detect when at rest upon the trunks of the cedars and arbor-vitæ which it frequents. The upper wings and exposed portions of the head and thorax are somber gray, the almost transparent wings showing a tinge of pink when spread. The under wings are either entirely black or have a small white spot near the center. The posterior dorsal portion of the abdomen is bright red or deep orange, the remaining portions of the body being black. The insect shows perfect protective coloration at rest and a rather typical warning combination in flight. The insects were eaten at once by the lizards when seen in motion.

COLEOPTERA.

Chauliognathus scutellaris Lec. Although this beetle is colored black and yellow it appears to be palatable. May 1 five were introduced. The first was taken by the lizard that sampled the *Lygæid*, tasted a little, and rejected. However three others were eaten by a second lizard and the last beetle by a third. May 4 twenty beetles were introduced and all were eaten without any evidences of unpalatability. On May 5 four were introduced just after the four *Lygæids*. The first was carefully examined before being eaten; the second was tasted and refused by another lizard; the others were not noticed, as was also the case when seven were introduced the following day. The lizards were probably too well fed, for since then, May 11 and 13, they have eaten all that were offered.

Epicauta sp. November 3. This black blister-beetle was tasted and rejected immediately. Unfortunately no more specimens were found.

Zopherus haldemani Sallé. This very hard Tenebrionid beetle, conspicuously colored in black and white, was introduced November 9 and removed alive December 13 during which time no attempt to take it was seen. Specimens experimented with November 12 and May 5 gave the same results.

Lucanus dama Thumb. This black, horny beetle was introduced November 17 and died January 7; during this time the lizards never tried to take it.

Harpalus caliginosus Fab. This beetle is large, black and rather hard, nevertheless one was eaten December 2, one December 12 and another partly eaten January 8. Four remained dead at this date. Their odor is offensive.

Brachynus sp. When seized this beetle ejects a strong, volatile acid with a sharp, audible report. This always surprised the lizards; nevertheless, of the four beetles placed in the cage three were eaten, but the last refused. Two more were introduced February 26 and one March 5, which afterward disappeared and presumably were eaten.

Brachynus sp. April 3. This beetle, larger than the preceding species, was eaten at once.

Calosoma angulatus Chev. and

Pasimachus depressus Fab. were introduced March 17. The lizards attempted to catch them, but failed, and soon gave up the chase.

Chlænius orbus Horn. The odor of this beetle is quite offensive. March 9 one was eaten at once. On March 10 two lizards tried to catch a specimen but failed repeatedly. They appeared to notice the odor and gave up the chase. On March 23, however, the lizard that ate the former now ate another, and still a fourth was eaten April 3, but with evident disgust.

Cantharis fulvipennis Lec. This large blister beetle has the typical warning colors of black and yellowish-brown and is further protected by a disagreeable secretion that exudes from the joints of the legs when the insect is seized and which is capable of producing blisters. Four of these beetles were introduced May 19 and each was seized at once, then quickly shaken off. The lizards eyed the beetles intently, but made no attempts to take them. These specimens were removed and introduced again the following day. Only one beetle was taken this time and it was quickly rejected. On May 21 several beetles were again introduced. One was caught and quickly rejected and no further notice was taken of them unless they crawled upon the lizards, in which case they were shaken off violently.

DIPTERA.

Musca domestica and *Stomoxys calcitrans.* A small lizard of this species (*Sceloporus floridanus*) soon became so tame that it

would lie on my hand and eat the flies which I caught and offered in my fingers. Sometimes he would catch the flies himself if I held him close to the window where they were crawling. He also ate a number of small spiders that were just emerging from the egg case placed in a glass jar. The lizard was kept in a cage with adults of the same species and was possibly eaten by them, as no trace of him could be found, and these lizards had, on two other occasions, been suspected of devouring small lizards.

HYMENOPTERA.

Pogonomyrmex barbatus var. *monefaciens*. These ants were eaten October 29, November 3, November 22 and May 24. The sting is quite severe.

Pachycondyla harpax, a stinging Ponerine ant, was eaten October 28.

Polistes annularis, a formidable wasp, was not noticed November 5.

ORTHOPTERA.

Gryllus abbreviatus. Several of these crickets were eaten March 7 and March 11. It is therefore probable that those introduced November 9, January 18, and January 19, were also eaten, since crickets seem to be a favorite food with all the species of lizards.

NEUROPTERA.

Panorpa nuptialis Gerst. This species has the wings of typical black and yellow warning colors. A female was introduced November 9 and a male November 15. Both disappeared in some way, but were not seen to be eaten.

ARACHNIDA.

Epeira fasciata Hentz. This protectively colored specimen was eaten October 25 and a second November 6.

SCORPIONS.

Centrurus caroliniensis Beauv. On March 23 the specimen which was introduced stung one of the lizards. He appeared to be in much pain and was so frightened at the scorpion that the experiment seemed likely to terminate there, but suddenly he seized the offending sting in his mouth and spitefully devoured the

whole specimen. The color of this scorpion would seem to afford it efficient protection. This, together with its flat form, frequently prevents its being noticed by a casual observer when the stone under which it rests is overturned.

MYRIOPODA.

Julus (*Spirobolus*) *multistriatus* Walsh. The specimen introduced November 15 was not molested, but when two were introduced February 12 a lizard bit off part of the head of one *Julus*. Both specimens died after a few days, neither being eaten. This myriopod has a hard integument and is defended by means of an acrid secretion that is thrown out from the repugnatorial glands along each side of the body. It has the habit of coiling up and remaining quiescent whenever it is touched. This action makes the lizards suspicious of it.[1]

EXPERIMENTS WITH GERRHONOTUS INFERNALIS BAIRD.

The favorite foods of these lizards are crickets, grasshoppers, spiders and scorpions. A few Hemiptera were eaten also.

LEPIDOPTERA.

Anosia plexippus Linn. April 1, April 2, April 4 (three). None of these specimens were eaten.

Papilio (*Lærtias*) *philenor* Linn. March 26, March 30, April 6. All were examined and rejected.

Pyrameis cardui Linn. November 17. Offered and refused.

Pyrrhanæa andria Scudder. November 9. Refused.

Colias eurytheme Boisd. March 30, April 1 (three), April 6. On the latter date the butterfly was taken by the wings but soon dropped, and all others were refused entirely.

[1] *Sceloporus floridanus* is badly infected with an interesting mite which attaches itself under the scales of the lizard until sexually mature and then crawls up on the wooden part of the cage to oviposit. The eggs are placed in a peculiarly constructed palisade and hatch as a six-legged larva that appears identical with the ordinary "red bug." The adult has a pubescent black integument; the head, anus and four pairs of legs are bright red. The legs are arranged in groups, two pairs being situated on the anterior portion of the body and two in the posterior region. Mr. Nathan Banks believes that this form may represent a new genus since it is the only lizard parasite that has been taken in this country, and appears to be closely related to the Italian genus *Geckobia*.

ORTHOPTERA.

Acridium americanum Scudd. November 15, November 24, January 28, March 11, March 30. This large grasshopper is of a very somber, dusty color and extremely quick in flight. Whenever introduced into the cage it was at once eaten eagerly. The lizard seized the insect by the thorax, held it thus for some time, regrasped it more anteriorly several times until the head was taken into the mouth. The insect was then swallowed slowly, the lizard chewing a while, pausing to rest, then gulping down another portion. On one occasion when the grasshopper became somewhat crooked, although it was nearly completely swallowed it was disgorged, straightened, and then devoured again.

Species unknown. On November 29 a large grasshopper was eaten in the usual way. The body, legs and head were dark green; the wings brown. The whole body was ornamented with white or yellow spots and lines.

Gryllus abbreviatus. December 12, January 10 (five), March 7 (several), March 10 (two), April 6 (several). All the specimens were eaten eagerly.

NEUROPTERA.

Panorpa nuptialis Gerst. November 9. Although this warningly-colored insect remained in the cage six days, no attempt was made to seize it.

COLEOPTERA.

Lucanus dama Thunb. November 8, was not eaten.

Zopherus haldemani Sallé. November 9, was refused.

Harpalus caliginosus Fab. December 11 and December 18. Five specimens were introduced, and all died.

Brachynus sp. February 12. Two of these beetles were introduced and were not noticed by the lizards, though offered repeatedly. They run very swiftly, hiding at every opportunity, and the lizards are probably too slow in their movements to catch so quick a prey.

Patrobus longicornis Say. The beetle was introduced February 13, and remained until March 5, but no attempt was made to take it.

Diabrotica punctata Oliv. February 13. These green-and-black beetles were probably too small for the lizards to perceive.

Chlænius orbus Horn. March 7. One of the lizards ran up to examine the beetle but when near turned aside, evidently discouraged because of the disagreeable odor, and did not try again to take it.

Pasimachus depressus Fab. March 17. The beetle was examined and refused.

Calosoma angulatus Chev. April 6. The beetle seemed never to have been noticed.

Chauliognathus scutellaris Lec. May 4. The lizards seemed to pay no attention to the beetle although fifteen specimens were introduced.

Cantharis fulvipennis Lec. Two specimens of this black-and-yellow blister beetle were introduced May 19. One was seized at once by one of the lizards, chewed a moment, then dropped quickly. The lizard began writhing and rubbing his mouth in the sand, appearing much distressed. The second beetle was not noticed by any of the lizards and was removed. On May 20 they eyed the beetle that was introduced, but made no attempt to take it. May 21, the specimen seemed not to be noticed. Others introduced May 26 gave the save negative result as the preceding experiment.

HEMIPTERA.

Brachymena myops. December 1, January 24. This protectively colored, malodorous form was not noticed by the lizards.

Lygæid. May 5. Two specimens of this warningly colored bug were introduced, examined and refused.

Fulgorid sp. November 5, November 6. Several specimens were eaten with evident relish. The bug was never refused if alive, but never eaten if dead.

HYMENOPTERA.

Polistes annularis. Linn. November 4, refused.

Camponotus sansabeanus Buckley. November 29 and *Camponotus festinatus* Buckley. April 13. These ants were possibly too small to be noticed.

ARACHNIDA.

Lathrodectes mactans. November 17, November 29, December 6 (two), December 18 (four), January 20 (two), February 2 (two), March 9, March 11, March 17, March 25, March 30, April 6, April 13 (four), April 20 (three), May 19 (two). These spiders are of a jet black color conspicuously marked with crimson or sometimes white, thus exhibiting striking warning coloration. They are even said to be poisonous, yet they were always quickly seized and eaten by these lizards.

Attus mystaceus. December 10, December 12 (two). The somber gray color of these spiders affords them good protection under the stones where they live. They were eaten eagerly.

Lycosa sp. March 9, March 23 (two), March 25. This spider resembles very closely in color the under side of the stones where it is often found. It was eaten at once when introduced.

SCORPIONS.

Centrurus caroliniensis Beauv. January 20, March 17, March 23 (two), April 13 (three), April 20 (six), April 27 (five), May 4, May 18 (six), May 19 (two), May 25 (two). All these specimens were eaten with evident relish and no attention was paid to the sting. The hard integument of the lizard prevents the penetration of the sting.

MYRIOPODA.

Julus (*Spirobolus*) *multistriatus* Walsh. The specimen was introduced November 18 and died January 7. It was not noticed by the lizards, as was also the case with two specimens introduced February 12.

EXPERIMENTS WITH CROTAPHYTUS COLLARIS Say.

Two of these lizards were captured November 9 and were not seen to eat a single insect until February 12. Various kinds of insects were placed in the cage, and though the lizards were quite tame and lively they would not eat. On January 23 a dish of water was placed in the cage and they learned to drink from the dish and also from the pipette used for refilling it. The water furnished their only nourishment for three months. A third lizard was captured April 4 and though very fierce at first,

became quite tame in about a week, allowing me to rub its head and body with my hand. These lizards occupied the cage with *Gerrhonotus infernalis* until December 1 when they were placed in a separate one. The experiments were as follows:

LEPIDOPTERA.

Meganostoma eurydice Boisd. December 15, was not eaten.

Papilio (*Lærtias*) *philenor* Linn. March 26, March 30. These were not noticed and were afterward removed. The specimen introduced April 2 was found dead and apparently unharmed the following day. On April 7 the specimen introduced the previous day was gone, and on April 8 the lizard last caught was seen eating a butterfly. On April 21 a specimen was introduced and only a part of the wings remained next day. However, the two specimens introduced on May 4 remained in the cage two days and were not eaten.

Colias eurytheme Boisd. March 30. The specimen was not noticed by the lizards and was removed next day. The two that were introduced April 1 were gone the day following and of the two introduced April 2 one was entirely eaten and only the torn wings of the second remained. On May 8 one of the lizards seized the specimen just introduced by the edges of the folded wings and ate it slowly, often pausing to rest, but never releasing it.

Anosia plexippus Linn. Introduced April 2. Next day the head and thorax were chewed up and one fore wing was missing. Others that were introduced afterward disappeared but were not seen when eaten. But on May 18 the butterfly was seized at once by one of the lizards and a second lizard bit off part of a wing. Between them they ate the specimen, but did not take the two introduced May 25.

Pieris occidentalis Reakirt. April 16, was eaten.

Grapta interrogationis Fabr. The specimen introduced April 27 was eaten, and those placed in the cage May 7 and May 9 were gone the following mornings. Probably they were also eaten.

Papilio cresphontes Cramer. April 23. This butterfly did not seem to be noticed by the lizards.

Anosia berenice var. *strigosa* Bates. This butterfly has the same warning coloration scheme as *Anosia plexippus*. It had disappeared next day and was probably eaten.

COLEOPTERA.

The following specimens were introduced, but none of them were eaten and were rarely ever noticed by the lizards, though offered repeatedly:

Harpalus caliginosus Fab. December 2.

Brachynus sp. February 13 (three).

Chlænius orbus Horn. March 7, March 23, April 3.

Micryxis distinctus Hald. March 7. This beetle was evidently too small for the lizards to perceive. They pay no attention to small insects, possibly because their eyes are not capable of perceiving them.

Chauliognathus scutellaris Lec. May 4 (eighteen), May 5 (six). All refused.

A notable exception to this custom of refusing beetles was seen when three black-and-yellow blister beetles, *Cantharis fulvipennis* Lec., were introduced May 19. A lizard seized one of the beetles and ate it, then seized a second. One of the other lizards tried to take it from the former, but was unsuccessful, and the second beetle was eaten. The third was apparently not noticed by any of the lizards and was soon removed. Specimens were introduced May 20, May 21 and May 26, but did not seem to be noticed.

Occasionally larvæ of beetles were introduced and eaten, but with the above exception these lizards do not appear to feed on imaginal Coleoptera. *Cantharis* probably does not appear in the natural habitat of the lizard, the latter being a mountain species, while the beetle is found in the fields on the Mexican poppy (*Argemone mexicana*).

ORTHOPTERA.

Gryllus abbreviatus. February 12 three specimens were introduced, one of which was dead, and was at once seized and eaten by a lizard. This was the first food it had taken since its capture, November 9, and it is the only instance known of a lizard eating a dead insect. The two remaining crickets disappeared

later and were evidently eaten. On March 23 one of the lizards tried repeatedly to catch one of the five crickets introduced, but failed, and finally gave up the chase, even refusing the insect when it was held before him in the forceps. The lizards were seen to catch and eat crickets on the following days: April 13 (two); April 20, April 27 (two), and on several occasions specimens that were introduced in the evening had disappeared by the following morning. Indeed, crickets seem to form the principal food of these lizards.

NEMOPTERA.

Panorpa nuptialis Gerst. December 12. This warningly-colored insect was apparently not noticed and died soon afterward.

DIPTERA.

Hermetia illucens Linn. December 13. This form resembles a wasp somewhat closely. It was not noticed by the lizards.

HEMIPTERA.

Lygæid sp. May 5. The lizards could not be induced to take the specimens.

HYMENOPTERA.

No experiments with Hymenoptera were made with these lizards.

ARACHNIDA.

Attus mystaceus. December 1. This spider was not noticed though offered repeatedly.

Lathrodectes mactans. Specimens were introduced January 20 (three), March 23 (two) but none were eaten.

Other small spiders (names unknown) were introduced at different times but were never eaten.

MYRIOPODA.

Scutigera forceps. December 18. Specimen refused.

SCORPIONS.

Centrurus caroliniensis Beauv. November 15. The scorpion stung one of the lizards and it seemed to suffer so intensely and was so frightened whenever the former came near it that the experiment was never repeated.

Three other species of lizards were placed in the same cage with *Crotaphytus collaris*, from which the following results were obtained:

1. *Cnemidophorus sexlineatus* Linn. One specimen was caught December 1 and died January 7 during which time it was never seen to take any food. This was also the case with two small lizards of this species that were in the cage with *Sceloporus*. They disappeared mysteriously and are supposed to have been devoured. The lizard is quite common, but difficult to catch, and it is regretted that more were not obtained for the experiments.

2. *Holbrookia texana* Trosch. Two of these lizards were placed in the cage early in April and have never been seen to take any food.

Eumeces sp. This small lizard was captured March 12. On March 30 it tore up and ate the body of a butterfly, *Pieris occidentalis* Reakirt. April 6 it caught, tore to pieces and ate a cricket larger in circumference than itself. April 8 it ate a large house fly and on April 10 a number of small mantids, *Stagmomantis carolina*, recently hatched. The lizard was very alert, spying the mantids at a distance of several inches, though the latter were quite small and exactly the color of the sand on the floor of the cage. On April 23 and May 8 other young mantids of the same size were eaten.

Phrynosoma cornutum Harl. The "horned toads" were kept in cages with other lizards and also separately and were never seen to eat anything but ants. They are especially fond of the large agricultural ant, *Pogonomyrmex barbatus* Smith var. *molefaciens* Buckley.

General Summary.

1. Only one instance is known of a lizard eating a dead insect.

2. Insects that move slowly do not attract the attention of the lizards so much as do the more active forms, hence those that remain quiescent are rarely even attacked.

3. Insects below a certain size are apparently not perceived by the large species of lizards. Examples of such insects are *Diabrotica punctata* Oliv., *Micryxis distinctus* Hald., and various ants (*Camponotus*).

4. Large beetles having hard elytra are seldom eaten.

5. A butterfly with mutilated wings was not taken for an hour and a half although another perfect specimen introduced at the same time was eaten at once.

6. If an insect (*e. g.*, a beetle) falls upon its back the lizards rarely ever seize it until it has gotten upon its feet again.

7. The myriopod *Julus* was not eaten by any lizard.

8. Although the combinations of black and yellow, black and orange, or black and red are supposed to serve the purpose of warning coloration, all insects possessing these colors were, at one time or another, eaten, with the possible exception of *Panorpa nuptialis* Gerst and a malodorous *Lygæid* bug.

9. *Sceloporus floridanus* is perhaps the most satisfactory lizard for these experiments since it eats insects of all groups.

10. *Sceleporus* seizes any part of the insect, but as a rule only the wings of the butterflies and large moths.

11. All the lizards except *Eumeces* seize the insect with the mouth and swallow it a little at a time, never biting off pieces, but keeping the insect entire. *Eumeces* swallows its prey thus if small, but when the insect is large he shakes and pulls it to pieces with his mouth and eats the separate pieces.

12. *Sceloporus* is very active and is not easily tamed.

13. *Gerrhonotus* is exceedingly slow in capturing its prey. It creeps up stealthily, pauses when quite near, examines the insect by protruding the tongue, rises as high as possible on the toes of the fore limbs and then seizes the insect by the back with a sudden spring. If the insect does not move it is frequently left unmolested. This lizard soon becomes quite tame but does not enjoy being handled. It was seen to drink water from the dish by lapping with the tongue, but usually preferred taking it from the pipette, allowing me to place a drop at a time on its outstretched tongue.

14. *Eumeces* sometimes drinks by *lapping* with the tongue, sometimes by sucking up the water. *Sceloporus*, *Crotaphytus* and *Phrynosoma* drink by *sucking* the water into the mouth. At first *Sceloporus* and *Crotaphytus* would drink only from the pipette, but were gradually induced to follow that to the dish and drink from the latter.

15. *Phrynosoma cornutum*, though apparently quite tame, seems at first rather shy about eating in confinement. Ants, especially the agricultural ants (*Pogonomyrmex*), are its only known food.

16. *Crotaphytus* is not accurate in seizing its prey. It often fails repeatedly and gives up the attempt.

17. The larger lizards were several times suspected of having eaten smaller specimens that had been placed in the same cage.

18. *Crotaphytus* soon becomes quite tame and enjoys being petted. The smaller ones crawled upon my hand in the cage and refused to be put down.

19. The largest *Crotaphytus* shed its skin during the night of May 6. Next morning the sand in the cage was very much dug out and heaped up, but no traces of the skin could be found.

20. A *Gerrhonotus* shed during the night of April 29. The old skin was turned wrong side out and probably came off nearly whole, though several parts were broken when it was found next morning. A second lizard shed May 22 and I watched it pull the old skin off wrong side out by creeping round and round the cage close to the sides. The skin was loosened first from the upper and lower jaws along the sides of the mouth, and began to peel off backward by the lizard's rubbing its head against the sand on the bottom of the cage.

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SEX RECOGNITION AMONG AMPHIPODS.[1]

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How do males of the amphipod crustacea distinguish the females? It is well known that the males of the Gammaridea have the curious habit of carrying the females under their body for a considerable time. This act of transportation has probably no further significance in relation to the fertilization of the eggs than to secure the proximity of the two sexes when the proper time for fertilization arrives. According to the observations of Della Valle on *Gammarus pungens* the eggs are not fertilized until after they are laid, oviposition occurring a short time after moulting. When the moulting of the female has been effected, the male bends his body beneath that of his mate and deposits spermatozoa upon the ventral surface of her thorax. The deposit of sperm is followed within half an hour by the laying of the eggs. After the act of copulation the male regains his original position and swims about with the female as before. The same relation of oviposition to moulting was found by Miss Langenbeck in *Microdeutopus*, the male leaving the female during her moulting process but soon resuming his previous position when the moult was completed.

The instinct of the male amphipod to seize and retain hold of the female is one of remarkable strength. The male retains his hold, despite all efforts to dislodge him, with remarkable persistence, and will still cling to the female after the posterior half of his body has been cut away. My own observations on the sexual behavior of amphipods relate mainly to three species, *Amphithoe longimana* Smith, *Hyalella dentata* Smith and *Gammarus fasciatus* Say. The sexual behavior of these three species is remarkably similar, athough they belong to as many distinct families. The female while being carried about keeps remarkably impassive. Her thoracic legs are drawn up, the abdomen held strongly flexed, the whole body assuming as compact a form as possible. She takes little or no part in swimming; the movement of the

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pleopods when the body is strongly bent upon itself serves only to keep a current of water passing by the gills. She is carried about like a helpless burden, allowing her vigorous spouse to assume the entire labor of transportation and the responsibility of keeping her as well as himself out of danger. The efforts of the male to seize the female and get her into the proper position to be carried have the effect of inducing her to throw herself into the characteristic bodily attitude and remain quiet. The attitude assumed by the female is similar to that observed in the ordinary thigmotactic reaction of amphipods and may, perhaps, be but the same form of response, somewhat modified and specialized in relation to the function of reproduction. When the males are torn away from the females they soon seize their partners again and roll them about into the proper position and then proceed on their way in apparent contentment. The female as soon as seized by the male curls up and allows herself to be rolled and tumbled about without a show of resistance or protest. The males, as a rule, are considerably larger than the females and usually get their partners into the desired position quite readily ; but when a small male attempts to carry a large female he experiences much difficulty. I have observed a male *Hyalella* endeavoring to carry a female somewhat larger than himself. After seizing the female he would turn her around until she finally came into the proper position for transportation, but owing to the larger size of his partner the male could not reach around her body so as to carry her away. No sooner was the female properly adjusted than the male would lose hold of her round body and the same efforts had to be repeated. During all this performance the female remained dutifully passive. After watching the further struggles of the male for over half an hour I became convinced, although he was not, that he had undertaken an impossible task, and discontinued my observations.

In order to ascertain if sight plays any part in sex recognition in *Hyalella*, I tore some males away from their partners, blackened their eyes with asphalt varnish, and placed them in a dish with several females. It was not long before each of the blinded males was provided with a mate. Sight, therefore, is not the determining factor in sex recognition in this species.

That the females are distinguished through the sense of smell seemed more probable, since it has been shown that among many insects sex recognition is brought about in this way. The sense of smell in crustaceans is often highly developed and in some groups probably affords the means by which the females are distinguished. The sense of smell in the crustacea is mainly, although not quite exclusively,[1] located in the first antennæ. To determine if the male distinguishes the other sex by this sense, recourse was had to the experiment which naturally suggested itself, of removing the first antennæ of several males and observing whether they experienced any difficulty in finding mates. It was found that after they had recovered from the slight shock of the operation, the males seized the females as eagerly as before and carried them about in the usual manner. Even after both pairs of antennæ were removed the females were seized and carried in the same way. It is very improbable, therefore, that the sense of smell plays an important part in enabling male Hyalellas to distinguish the other sex. The experiment was then tried of placing several females in a small enclosure of wire gauze, while several males which had recently been torn from females were placed in the same dish, but outside of the enclosure. The males paid not the slightest attention to the females within the gauze; but soon after the gauze was raised and the females allowed to scatter through the dish most of the males had acquired a partner.

If one attentively observes Hyalellas as they are swimming about, it will be seen that the males do not pursue the females, great as their eagerness may be to seize and carry one of the opposite sex. Only when the two sexes collide in their apparently random movements does the male become aware of the presence of the female. When a male and a female collide, the female curls up and lies quiet while the male makes efforts to seize her. Should two females collide, they may curl up for a moment, but as they are not seized they soon pass on. When two males meet there is often a lively struggle. Each apparently attempts to seize and carry the other, but as neither will consent to remain passive they soon separate. The different reactions of the two sexes to

[1] Bethe, *Archiv. mic. Anat.*, Bd. 2, 1897; Holmes, BIOL. BULL., Vol. II., 1901.

contact with other individuals is the factor which effects the union of the males with the females. Each reacts to the reactions of the other. The male has a strong instinct to seize and carry other individuals of the same species. The female has the instinct to lie quiet when another individual comes into contact with her, especially if she is seized. The instinctive reactions of the two sexes are complementary and coöperate to bring about and maintain the peculiar sexual association characteristic of the Gammaridea. If the association of the sexes is brought about by their peculiar modes of reaction to certain contact stimuli, it would seem probable that the only reason why males do not carry other males as well as females is that they are prevented from so doing by the active resistance of their intended mates. I was accordingly led to try the experiment of mutilating some male specimens so that they could no longer make effective resistance to seizure. The large second gnathopods (the principal means of defense) of several males were cut off and the mutilated individuals were placed in a dish with several males which were recently torn from females. The mutilated males were soon seized and carried about as if they were members of the other sex. In one case a mutilated male was carried about for over five hours. The mutilated males were more active than females are under the same conditions, and did not assume the same bodily attitude, but nevertheless their captors carried them without any manifest awareness of the deception to which they were subjected.

Male Hyalellas, however, will not carry dead specimens of either sex, at least for more than a short time. I have observed males of both *Hyalella* and *Gammarus* struggling for a time with a dead specimen, but their efforts to carry it were soon discontinued. The failure to carry dead individuals may be due to odor or some sort of chemical stimulation from the object seized, or to the lack of an occasional movement causing a struggle on the part of the male to retain his hold. Stimuli of the latter kind may be necessary to cause the instinctive reaction of the male to continue.

There can be little doubt that the origin of the instinct of the male amphipod to seize and carry the female is to be sought in a modification of the act of copulation. The lower crustacea af-

ford many cases in which the association of the two sexes is prolonged for a considerable period. The males of *Artemia* clasp the females with their peculiarly modified antennæ and the two sexes swim about together for several days (Leydig). Among the free-swimming copepods the male may continue clasping the female for some hours after, as well as before, depositing the spermatophore (Jurine, von Siebold). And among the Cumacea Dohrn has observed the males swimming about upon the backs of the females, much as in Amphipoda. The tendency for the association of the sexes greatly to exceed the act of copulation is apparently quite widespread among the crustacea ; and although, so far as is known, the mating instinct of the Gammaridea is much the same throughout the group so that we cannot trace the successive steps in its development, the sexual behavior of some of the lower crustacea presents many features which may serve to throw some light upon its origin.

REGENERATION OF THE LEG OF AMPHIUMA MEANS.

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My object in studying the regeneration of the limbs of *Amphiuma means* was to discover whether the limbs, which appear to be of so little use to the animal as organs of locomotion, have the power to regenerate as have the limbs of other urodele amphibia.

The first amphiuma that I obtained (in 1900) was a large individual, and after several months had begun to regenerate, but died as the result of an accident before regeneration had gone very far.[1] The next individual that I was able to procure was also large, but escaped before regeneration had gone any farther than in the last case. Two smaller individuals have been kept for more than a year (from March 21, 1901, to May 3, 1902). The following account applies to them. Each had a fore-leg and hind-leg of opposite sides cut off through the upper portion of the leg. In the course of several weeks a knob of new tissue appeared which continued to elongate for several months, when further growth seemed to have ceased. To make certain of this, the animals were kept for six months longer, but no further change occurred. The new part was shorter than the part removed, and appeared to be a single rod, tapering at the end, without any external signs of toes.

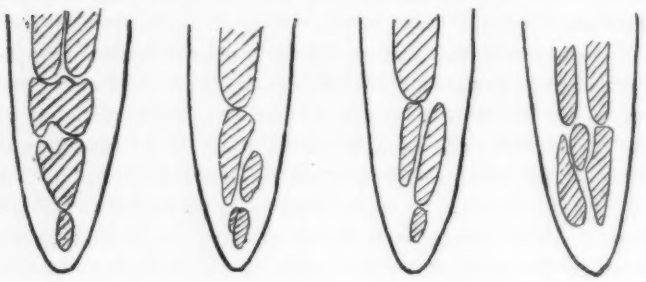
The normal fore- and hind-foot of the amphiumas that I used had each three toes. Cope[2] gives a figure of the skeleton of amphiuma showing a cartilaginous carpus of four or five pieces, and three ossified metacarpals with ossified phalanges. In the hind-foot there are three cartilaginous tarsalia, three ossified metatarsals and three phalanges.

After the legs had regenerated they were cut off, imbedded in paraffine, and cut into sections. These showed in three of the four cases that the two bones of the middle part of the limb have

[1] This is the case referred to in Towle's paper. BIOLOGICAL BULLETIN, II., 1901.

[2] Cope, "The Batrachia of North America," Bull. U. S. Nat. Mus. No. 34.

developed. The condition of the carpus and tarsus appears to be different in each of the four cases, Figs. 1–4. The rough reconstructions shown in these figures were made from sections. The figures are not very accurate, but serve to show the number of bones and their relation to each other. The relative sizes of the bones is less exact. It will be seen from the figures that the regeneration has lead neither to the formation of a uniserial row of skeletal elements, nor is it clear in all cases whether more than a single toe is represented. It seems probable that the



terminal middle phalanx represents a toe, but whether any of the other cartilages represent other suppressed toes can not be stated.

In these four cases the legs had been cut off through the humerus, or the femur. It occurred to me that if the limb were cut off through the fore-arm or the fore-leg the result might possibly be different, since two bones are present at the cut surface. Therefore on May 3, 1902, when the two regenerated legs were removed for study, the remaining two legs were cut off through the fore-leg and fore-arm.

The two amphiuma were kept alive for nearly another year; until March 30, 1903. They were occasionally fed on earthworms. The limbs that had been cut off through the fore-arm and fore-leg regenerated, but again produced only a single pointed, or in one case a somewhat flattened, new part. Serial sections show that, besides completing the ends of the two bones at the exposed surface, there have been produced a number of more distal cartilages. The arrangement of these pieces is irregular, and different in each case, as also occurred when the leg was

cut off through the upper portion. In other words, no better regeneration took place here than in the former instances.

It is also of interest to notice that the other two legs that had been cut off (close to the body) for examination had not regenerated. The skin grew over the cut surface, and in several cases the muscles of the body wall seemed to have grown over the short piece of the humerus or femur that had been left. At most, a short protrusion indicated the position of the limb.

How shall we interpret this result. Those who hold that the power to regenerate a part is commensurate with the value of the part to the animal, if it is a part liable to injury, will welcome this experiment as in harmony with their interpretation. On the other hand, as I have tried to show elsewhere, the evidence is so strong against this point of view that I think we shall not go wrong if in this case we deny that the result has any such meaning.

In fact, in other adult amphibia, in the frogs for instance, in which the limbs are of some importance to the animal they cannot be regenerated, although in the tadpole stage in which the limbs are of no importance, and, in the case of the fore-limb at least, not liable to injury, the power of regeneration is present. Moreover even in the urodeles the power of regeneration is unequally developed in forms that use their legs for purposes of locomotion. It is said that *Triton marmoratus* shows only a slight power to regenerate its legs. In other cases, as I have observed in *Necturus*, the time required to regenerate a leg is so long that were the presence of the leg essential to the existence of the individual it would succumb before the regeneration could take place.

These considerations make it clear, in my opinion, that the lack of complete power to regenerate in amphiuma can not be interpreted as having any connection with the unimportance of the legs to the animal. It should not be overlooked that it is not that the leg does not regenerate at all; in fact it regenerates quite well, but that the new part is different from the old. It is at least conceivable that some simple physical or physiological factor may interfere with the formation of the complete toes, such, for instance, as the thickness of the skin in relation to the size of the limb.

If it could be shown that the leg of amphiuma is a degenerate structure it might appear that there is some connection between the degeneracy of the part and its lack of power to regenerate, but it is far from being established that any such general relation really exists. In fact, in the male hermit crab I found that the very small and *apparently* rudimentary abdominal appendages have the power to regenerate. It would be interesting, nevertheless, to examine this point further in cases where the degeneration and uselessness of an organ are more certainly established, as in the case, for example, of the appendix of man, which does not appear to have the power to regenerate after removal.

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